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Talinum paniculatum (Jacq) Gaertn (Java ginseng) Production using Vesicular-Arbuscular Mycorrhizal

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Abstract

Soil pathogen is one of main problem in Java ginseng production in tropical field. This study was aimed to evaluate the impact of VAM application to Java ginseng tuber production. Application of VAM was conducted in various concentrations (0, 1, 2, and 3%) (w/w). Thin Layer Chromatography was used to detected saponin and spectrophotometry was used to determine total saponin. Biomass, the number of rotten tuber, and total saponin were determined after eleven months under greenhouse condition. Inoculation 3% of VAM can enhance the number of healthy and biomass Java ginseng tuber significantly. But the application of VAM did not increase total saponin content.

Article History

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Keyword

Saponin
Vesicular-Arbuscular
Mycorrhizal
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Introduction

Java ginseng (Portulacaceae) is an herbaceous medicine of tropical plant. The plant is widely used as active compounds source, such as saponins, flavonoids, tannins, triterpenes or sterols, and polyphenols. Java ginseng has been known for its effectiveness as inducer on spermatogenesis and motility of sperm, anti-inflammatory, androgenic potency, and sperm viability (Saroni *et al.*, 1999; Sumastuti, 1999; Winarni, 2009; Rahmi *et al.*, 2011). Saponins are glycosylated secondary metabolites distributed in plant tissues (Hu *et al.*, 2003). Santoso *et al.* (2016) was reported that Java ginseng rich of Ginsenosides, an important triterpenoid saponin as well as the other ginsengs. Java ginseng is a potential medicinal plant with activity as good as other medicinal plants such as Korean ginseng or another ginseng from other countries.

There are many previous studies revealed the efficacy of Java ginseng, but its production in Indonesia has not been optimal yet. There are some factors which inhibit Java ginseng production in Indonesia, such as the lack information of good cultivation techniques in field. Java ginseng plantation in field also has its own some challenges, such as tuber-eating nematode which attack the plant and cause tuber rotten as well as soil pathogens do. This

case has been recorded on Sewon Bantul Yogyakarta and Plosoklaten Kediri (East Java Province) Indonesia as the central of Java ginseng production.

Vesicular-Arbuscular Mycorrhizal (VAM) is vesicle formed by fungi which have a benefit on plant growth (Sikes, 2010; Gazey *et al.*, 2004; Al-Karaki & Al-Raddad, 1997) because it can absorb phosphorus in optimum amount plants needed to survive in drought condition (Nurhayati, 2010; Berta *et al.*, 1995). It also protects plants from pathogens (Rianto *et al.*, 2005; Gazey *et al.*, 2004; Simanungkalit, 1997). For example, a *Glomus sp.* fungus on VAM has capability in inhibition of several microbial pathogens activity which causes plant wilting, such as *Pseudomonas solanacearum* (Rianto *et al.*, 2005). Plant with mycorrhizal roots can absorb Zn and S from the ground faster than plant with non-mycorrhizal root (Ruiz-Lozano *et al.*, 1995; Abbott *et al.*, 1992). This means that VAM application is promising in increasing the Java ginseng production in Indonesia.

One problem of Java ginseng tuber production in Indonesia is the lack information of good planting techniques. This research is important because the results will help to obtain information about good planting techniques which can be applied by farmers to provide healthy tuber of Java ginseng.

Materials and Methods

Application of VAM

Java ginseng seeds were collected from field in Plosoklaten Kediri East Java during dry season from May to August 2013. Java ginseng seed were obtained by cutting the dry flower panicles. Seeds were germinated in tray and taken at 21 days after germination. Germinated Java ginseng then transferred into poly-bags filled with soil and sand (1:1) and planted for 2 weeks. The seedlings then inoculated with VAM in some concentration (0, 1, 2, and 3%) (w/w). VAM inoculates which used in this research were obtained from Biotechnology Center, Bogor Agricultural Institute. Plants were treated for 8 months with liquid compost supplementation. Weeding and pest controlling were done during the treatment. Java ginseng roots were harvested by cutting the stem base at 3-4 cm from the surface of planting medium. Planting medium with root was given water until saturated then the plastic bag was cut on one side and root can be released slowly. Furthermore, ginseng roots were cleaned from soil particles using tap water. Roots were obtained biomass under 70°C until constant condition (g). Healthy tubers per plant was evaluated by counting of rotten bulb using this formula (rotten bulb index = $[NR/NHR]$; NR is the number of rotten bulb of tuber per plant and NHR is the number of healthy tuber per plant)

Determination of Saponin Content

Saponin content of air dried Java ginseng bulbs were measured through qualitative, semi quantitative and quantitative methods (Santoso, 2012). One gram of dried samples was grinded into fine powder in room temperature. Extraction was done by maceration using 80% of ethanol for 3 times then saturated in water bath on 80°C until shrunk into 0,1 mL. Detection of Java ginseng root saponin content has been done using Thin Layer Chromatography (TLC) method using propanol and water (14:3). Chromatograms then was removed, dried at room temperature, and sprayed with anisaldehyd-H₂O₂, followed by heating at 110°C. The saponin existence will be presented by dark green to black spot. While quantitative test performed by spectrophotometer (365 nm) with 80% of ethanol which reacted with vanillin reagent. TLC and quantitative test were calculated according to the method as described by Santoso

(2012). All chemical reagents were purchase in pro analysis grade by Sigma.

Data Analysis

Biomass and total saponin were performed by one-way analysis of variance (ANOVA) using the statistical software SPSS for *Windows*. Duncan's multiple range tests were used to evaluate significant difference between means at a significant level ($P < 0.05$). Biomass and saponin spot in chromatogram were evaluated descriptively.

Results and discussion

VAM inoculation was conducted in Java ginseng successfully for 8 months under greenhouse condition. Application of VAM can enhance of biomass (Table 1) and the number of healthy Java ginseng tuber (Table 2). There were a significant increase of biomass and the number of healthy Java ginseng tuber. In the present research. Higher biomass can be obtained by adding 3% of VAM ($11,11 \pm 0,49$ g per plant) but inoculation of 2% can provide healthy tuber of Java ginseng. In control group, the number of rotten bulb up to 28% (0,28) (Figure 1B) and tended to decrease in 1% VAM inoculation.

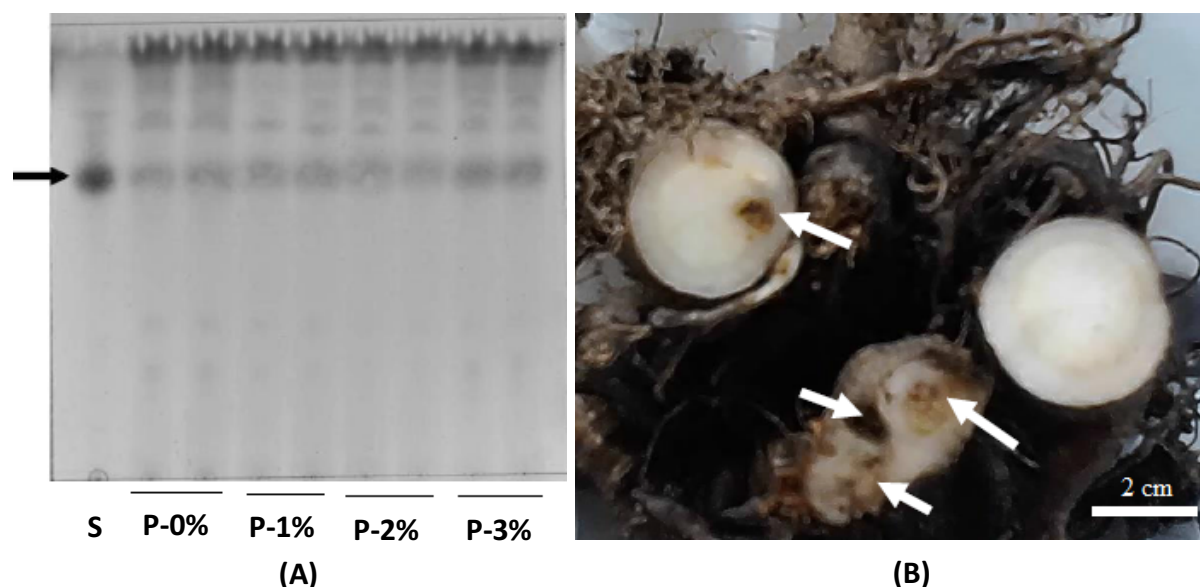


Figure 1. Thin Layer Chromatography of Ethanol Extract of Java Ginseng Tuber after VAM Inoculation on Silica Gel (standard saponin = S) (A) and spot of rotten bulb in control group caused pathogen (white arrow) (B).

Table 1. Biomass of Java Ginseng tuber after VAM inoculation (g)

Treatment	Biomass (g)
P-0%	$9,16 \pm 1,42^c$
P-1%	$10,21 \pm 0,84^a$
P-2%	$10,61 \pm 0,78^a$
P-3%	$11,11 \pm 0,49^b$

*Different letters in the same column indicate significant differences at the level of Duncan test ($P < 0.05$)

In order to evaluate saponin content, TLC was used and be confirmed quantitatively by using spectrophotometry method. Both of TLC and spectrophotometry method were done successfully. All samples contain rich saponin. It can be confirmed by the same color of spot

which appear on chromatograms as the color of saponin standard (Figure 1A). Thus, the result indicated that there was no significant total saponin of Java ginseng tuber after VAM inoculation (Table 3). The present study revealed that VAM inoculation in Java ginseng can enhance the biomass and improving the healthy tuber but did not total saponin.

Table 2. Root number of Java Ginseng on 8 months after VAM inoculation.

Treatment	Rotten bulb
P-0%	0,28
P-1%	0,11
P-2%	-
P-3%	-

*Rotten Parts

Table 3. Saponin content on Java Ginseng tuber (mg/g sample)

Treatment	Saponin Content
P-0%	13,33 ± 0,3055 ^a
P-1%	13,34 ± 0,8083 ^a
P-2%	13,23 ± 0,5133 ^a
P-3%	13,37 ± 0,3055 ^a

*Different letters in the same column indicate significant differences at the level of Duncan test ($P < 0.05$)

VAM application on plant is expected to give benefit on plant growth (Berta *et al.*, 1995; Michelsen & Rosendahl, 1990). The benefit for plant is VAM can provides some essential nutrients such as P, Mg, K, Fe, and Mn (Setiadi, 1989). VAM has ability to form colony as mycorrhizal hyphae on root surface (Rianto *et al.*, 2005; Al-Karaki *et al.*, 2004; Berta *et al.*, 1995). This hyphae extension helps root in nutrients intake, especially in nutrient-poor conditions, low pH, and lack of water (Dearnaley, 2007; Stewart *et al.*, 2005). Mycorrhizal on plant roots can increase Zn and S absorption from ground faster than non-mycorrhizal root plants (Abbott *et al.*, 1992; Michelsen & Rosendahl, 1990). Application of VAM does not increase plant secondary metabolites production, such as saponin in Java ginseng tubers in this study. The mechanism of saponin biosynthesis or main function in plant are unclear until now (Santoso *et al.*, 2016).

On the other hand, VAM application prevents rotten tuber. Rotten tuber can be detected by cut the tuber transversely. Healthy tubers do not have any spots on it when being cut transversely. Brown or red spots found in tuber when being cut indicate infection that can spread and root will become rotten. This result study shows that VAM application can prevents tuber damage caused by soil pathogens attack (Yadav *et al.*, 2012; Sikes, 2010; Berta *et al.*, 1995), such as fungi and nematodes (Michelsen & Rosendahl, 1990). Previous study revealed that the existence of several fungi such as *Glomus sp* (Rianto, *et al.*, 2005; Al-Karaki *et al.*, 2004; Al-Karaki, 1998; Al-Karaki & Al-Raddad, 1997). Further research needed to be done specially to evaluate the nutrient uptake, physiology, and ginsenoside kinetics of Java ginseng under VAM inoculation in field

Conclusions

This study shown that VAM application on java ginseng prevents tuber damage caused by pathogen. However, there is no significant difference on dry biomass data and saponin levels of Java ginseng tuber between control and treatment group. Therefore, further

research about the increasing of saponin level is needed, such as drought stress techniques.

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